Listing of Claims

1. (Currently amended) A method of analyzing the protein content of a population of cells from a tissue sample, comprising:

extracting the population of cells from the tissue sample using microdissection under microscopic visualization;

isolating a protein sample from the extracted cell population; and

analyzing the isolated protein sample.

2. (Currently amended) The method of claim 1 wherein isolating the protein sample comprises solubilizing the extracted cell contents in ~~a small volume~~less than about 20 µl of a buffer comprising at least one detergent to solubilize the cellular lipids, at least one proteinase inhibitor to preserve protein content and function, and at least one salt to lyse the nuclear contents.

3. (Currently amended) The method of claim 2 wherein the cell contents are solubilized in ~~small volume of buffer is~~ about 1µl to about 15 µl of buffer.

4. (Currently amended) The method of claim 1 wherein analyzing the isolated protein sample comprises performing a soluble immunoassay using a labeled antibody specific for a protein of interest.

5. (Original) The method of claim 4 wherein the labeled antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactivity.

6. (Currently amended) The method of claim 1, wherein the method is a method of quantifying the amount of a protein of interest in a population of cells,

wherein extracting the population of cells from the tissue sample comprises laser capture microdissection; and

wherein isolating the protein sample from the extracted cell population comprises solubilizing the extracted cell contents in about 1µl to about 15µl of a buffer where the buffer comprises Tris-HCl, NP-40, sodium deoxycholate, sodium chloride, EDTA, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and AEBSF; and

wherein analyzing the isolated protein sample comprises performing a soluble immunoassay using an antibody specific for a protein of interest in the isolated protein sample, where the antibody is

labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactivity, and ~~wherein~~ calibrating the assay ~~is calibrated~~ to indicate the amount of the protein of interest present in the isolated protein sample.

7. (Currently amended) The method of claim 6 wherein the protein of interest in the isolated protein sample is prostate soluble antigen (PSA).

8. (Currently amended) The method of claim 1 wherein analyzing the isolated protein sample comprises:

performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins in the isolated protein sample from each other; and

further analyzing the proteins in the isolated protein sample using a protein specific dye or Western blotting with a labeled antibody specific for the protein of interest in the isolated protein sample.

9. (Currently amended) The method of claim 1 wherein analyzing the isolated protein sample comprises

performing a two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate the proteins in the isolated protein sample from each other;

isolating a protein of interest from the gel; and

determining an amino acid sequence of the protein of interest.

10. (Original) The method of claim 9 wherein the sequence is determined using a method selected from the group consisting of N-terminal sequencing, mass spectrometry MS-MS sequencing, liquid chromatography quadrapole ion trap electrospray (LCQ-MS), and matrix assisted laser desorption/time of flight analysis (MALDI/TOF).

11. (Currently amended) The method of claim 1 wherein analyzing the isolated protein sample comprises performing surface enhanced laser desorption ionization spectroscopy (SELDI) to produce a protein fingerprint for the cell population.

12. (Original) The method of claim 1 wherein the cell population is microscopically identifiable as a tumor cell.

13. (Previously presented) The method of claim 1, wherein the method is a method of characterizing binding properties of one or more intracellular proteins of a population of cells, wherein analyzing the isolated protein sample comprises:

performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate the proteins from each other;

removing at least one protein of interest from the gel;

further analyzing the protein of interest by incubating the protein with a known or putative binding partner for the protein of interest; and

determining whether the protein of interest binds to the known or putative binding partner.

14. (Original) The method of claim 13 wherein the protein of interest is PSA and the known binding partner is alpha-1-antichymotrypsin (ACT).

15. (Previously presented) The method of claim 1, wherein the method is a method of differentiating a protein content of at least two populations of cells of a tissue sample, comprising:

extracting at least a first and a second population of cells directly from one or more tissue samples using laser capture microdissection;

isolating protein from the extracted cell populations to generate for each cell population an isolated protein sample having a content;

analyzing the isolated protein sample for at least two cell populations; and

comparing the protein content of the isolated protein sample of at least the first cell population to the protein content of the isolated protein sample of at least the second cell population to identify differing content.

16. (Currently amended) The method of claim 15 wherein isolating protein comprises solubilizing the extracted cellular material in ~~a small volume~~less than 20 µl of a buffer wherein the buffer comprises Tris-HCl, ~~NP-40~~NONIDET® P40 (octylphenolpoly(ethyleneglycolether)), sodium deoxycholate, sodium chloride, ethylenediaminetetraacetic acid~~EDTA~~, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-bezenesulfonylfluoride~~AEBSF~~.

17. (Currently amended) The method of claim 15 wherein the cell contents are solubilized in ~~small volume of buffer is~~ about 1µl to about 15 µl of buffer.

18. (Previously presented) The method of claim 15 wherein analyzing the isolated protein comprises performing a soluble immunoassay using a labeled antibody specific for a protein of interest wherein the assay is calibrated to indicate the amount of the protein of interest present in the sample.

19. (Currently amended) The method of claim ~~15~~ 18 wherein the immunoassay is of high sensitivity and the labeled antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactive labels.

20. (Previously presented) The method of claim 15 wherein analyzing the isolated protein comprises:

performing a two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins from each other;

isolating a protein of interest from the gel; and

determining an amino acid sequence of the protein of interest.

21. (Original) The method of claim 20 wherein the sequence is determined using a method selected from the group consisting of N-terminal sequencing, mass spectrometry MS-MS sequencing, liquid chromatography quadrapole ion trap electrospray (LCQ-MS), and matrix assisted laser desorption/time of flight analysis (MALDI/TOF).

22. (Previously presented) The method of claim 15 wherein analyzing the isolated protein comprises:

performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate protein fractions from each other; and

further analyzing the protein fractions using a protein specific dye or Western blotting with a labeled antibody specific for a protein of interest.

23. (Original) The method of claim 15 wherein the first population of cells and the second population of cells are from the same tissue sample and the first population is microscopically identifiable as tumor cells and the second population is microscopically identifiable as normal cells.

24. (Original) The method of claim 15 wherein the first population comprises several subpopulations wherein each subpopulation is microscopically identifiable as cells at different stages of tumor progression.

25. (Currently amended) The method of claim 1, wherein the method is a method of comparing the protein content of a first population of cells microscopically identifiable as tumor cells to the protein content of a second population of cells that are normal wherein both populations of cells are extracted from the same tissue sample, the method comprising:

extracting the first and second populations of cells from the tissue sample using laser capture microdissection, in which a laser targets the first and second populations as microscopically distinct and separates them from a larger microscopic structure; and

isolating a protein sample from each extracted cell population by solubilizing the extracted cell contents in about 1µl to about 15 µl of a buffer where the buffer comprises Tris-HCl, NONIDET® P40~~NP-40~~ (octylphenolpoly(ethyleneglycolether)),, sodium deoxycholate, sodium chloride, ethylenediaminetetraacetic acid~~EDTA~~, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-bezenesulfonylfluoride~~AEBSF~~;

wherein analyzing each of the isolated protein samples comprises:

performing a one dimensional polyacrylamide gel electrophoresis ~~(1D PAGE)~~ or two dimensional polyacrylamide gel electrophoresis ~~(2D PAGE)~~ to separate proteins of the protein sample from each cell population;

further analyzing the separated proteins of each cell population using a protein specific dye or Western blotting with a labeled antibody specific for a protein of interest; and

comparing a protein of interest content of the first cell population to a protein of interest content of the second cell population.

26. (Currently amended) The method of claim 1, wherein the method is a method of comparing the protein content of a first population of cells microscopically identifiable as tumor cells to the protein content of a second population of cells in order to identify the origin of the first population of cells, the method comprising:

extracting the first and second populations of cells from the tissue sample and from each other using laser capture microdissection;

isolating a protein sample from each extracted cell population by solubilizing cells from extracted cell populations in about 1 µl to about 15 µl of a buffer where the buffer comprises Tris-HCl, NONIDET® P40~~NP-40~~ (octylphenolpoly(ethyleneglycolether)), sodium deoxycholate, sodium chloride, ethylenediaminetetraacetic acid~~EDTA~~, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and 4-(2-aminoethyl)-bezenesulfonylfluoride~~AEBSF~~; and

wherein analyzing each of the isolated protein samples comprises:

performing surface enhanced laser desorption ionization spectroscopy (SELDI) to produce a protein fingerprint of the protein sample for each cell population; and

comparing the protein fingerprint of the first population of cells to the protein fingerprint of a known second population of cells to determine whether or not the two populations have the same origin.

27. (Original) The method of claim 26 wherein said first population of cells is microscopically identifiable as a tumor metastasis and the second population of cells is one of a battery of known normal tissue samples.

28. (Original) The method of claim 27 wherein the known normal tissue samples are from the same patient as the first population of cells.

29-33. (Cancelled)

34. (Previously presented) The method of claim 1, wherein the method is a method of screening for the presence of a cellular component in a population of cells from a tissue sample, wherein isolating the protein sample from the extracted cell population comprises:

lysing the extracted cell population to produce cellular components;

wherein analyzing the isolated protein sample comprises:

immobilizing at least one cellular component or a binding agent in a confined zone;

contacting the cellular components with a binding agent; and

detecting the interaction between the components and the binding agent.

35. (Original) The method of claim 34 wherein the cellular component or the binding agent is labeled, and detecting the interaction between the cellular component and the binding agent comprises detecting the presence of the label.

36. (Previously presented) The method of claim 35 wherein the label is detected by a method selected from the group consisting of a colorimetric, chemiluminescent, radioactive, and fluorescence.

37. (Original) The method of claim 34 wherein the confined zone of the immobilized cellular component or the immobilized binding agent is an array.

38. (Original) The method of claim 34 wherein the cellular component is immobilized.

39. (Original) The method of claim 34 wherein the binding agent is immobilized.

40-43. (Cancelled).

44. (Previously presented) The method of claim 1, wherein analyzing the isolated protein sample comprises generating on a substrate an array comprising a series of at least two dilutions of the protein sample.

45. (Previously presented) The method of claim 44, wherein analyzing the isolated protein sample further comprises:

applying a first labeled probe that specifically detects a first protein analyte; and

obtaining a quantitative value for the first protein analyte by comparing a signal from the first labeled probe at different positions in the dilution series.

46. (Previously presented) The method of claim 45, further comprising:

applying a second labeled probe that specifically detects a second protein analyte; and

obtaining a quantitative value for the second protein analyte by comparing a signal from the second labeled probe at different positions in the dilution series.

47. (Previously presented) The method of claim 6, wherein calibrating the assay comprises generating a serial dilution of the protein sample.

48. (Previously presented) The method of claim 15, wherein analyzing the isolated protein sample for at least two cell populations comprises generating on a substrate an array comprising a series of at least two dilutions of each protein sample.

49. (Previously presented) The method of claim 48, wherein analyzing the isolated protein sample for at least two cell populations further comprises:

applying a first labeled probe that specifically detects a first protein analyte; and

obtaining a quantitative value for the first protein analyte by comparing a signal from the first labeled probe at different positions in each of the dilution series.

50. (Previously presented) The method of claim 49, further comprising:

applying a second labeled probe that specifically detects a second protein analyte; and

obtaining a quantitative value for the second protein analyte by comparing a signal from the second labeled probe at different positions in each of the dilution series.

51. (Previously presented) The method of claim 18, wherein calibrating the assay comprises generating a serial dilution of the protein sample.

52. (Cancelled).

53. (Previously presented) The method of claim 37 wherein the cellular component is immobilized.

54. (Previously presented) The method of claim 34, wherein the confined zone is a microspot on a microarray.

55. (Previously presented) The method of claim 1, wherein the isolated protein sample is referred to as a first isolated protein sample, further comprising analyzing the protein content of at least a second population of cells from the tissue sample, or from a second tissue sample, which method comprises:

extracting a second population of cells from the tissue sample or the second tissue sample;

isolating a second protein sample from the second extracted cell population; and

analyzing the second isolated protein sample concurrently with the first isolated protein sample.

56. (Previously presented) The method of claim 55, wherein the protein contents of more than two populations of cells are analyzed.

57. (Previously presented) The method of claim 56, wherein the more than two populations of cells are extracted from more than two tissue samples.

58. (Previously presented) The method of claim 56, wherein the more than two populations of cells are extracted from:

tissues from different stages of malignancy;

tissues before and after a treatment;

tissues from different stages of development of an embryo; or

combinations thereof.

59. (Previously presented) A method of analyzing the protein content of more than one population of cells from at least one tissue sample, comprising:

extracting the more than one population of cells from the tissue sample(s);

isolating a protein sample from each of the extracted cell populations; and

analyzing the isolated protein samples.

60. (Previously presented) The method of claim 59, wherein the cells are extracted from more than one tissue sample.

61. (Previously presented) The method of claim 60, wherein the more than one tissue samples are from a single subject.

62. (Previously presented) The method of claim 59, wherein extracting the more than one population of cells from the tissue samples comprises using microdissection.

63. (Previously presented) The method of claim 62, wherein the microdissection comprises laser capture microdissection.

64. (Previously presented) The method of claim 59, wherein the more than one population of cells extracted from the tissue sample(s) is cultured *in vitro* prior to the step of isolating the protein sample from each of the cell populations.

65. (Previously presented) The method of claim 64, wherein the more than two populations of cells are extracted from:

tissues from different stages of malignancy;

tissues before and after a treatment;

tissues from different stages of development of an embryo; or

combinations thereof.

66. (Previously presented) The method of claim 59, wherein the method is a method of screening for the presence of a cellular component in the more than one population of cells, wherein isolating the protein sample from each of the extracted cell populations comprises:

lysing the extracted cell populations to produce cellular components;

and wherein analyzing the isolated protein sample from each of the extracted cell populations comprises:

immobilizing at least one cellular component or a binding agent in a confined zone;

contacting the cellular components with a binding agent; and

detecting the interaction between the components and the binding agent.

67. (Previously presented) The method of claim 66 wherein the cellular component is immobilized.

68. (Previously presented) The method of claim 67 wherein the confined zone of the immobilized cellular component or the immobilized binding agent is an array.

69. (Previously presented) The method of claim 66, wherein the confined zone is a microspot on a microarray.

70. (Previously presented) The method of claim 1 wherein analyzing the isolated protein comprises performing an immunoassay using a labeled antibody specific for a protein of interest, wherein the assay is calibrated to indicate the amount of the protein of interest present in the sample.

71. (Previously presented) The method of claim 70, wherein calibrating the assay comprises generating a serial dilution of the protein sample.

72. (Previously presented) The method of claim 34 wherein analyzing the isolated protein further comprises using a calibration to indicate the amount of the protein of interest present in the sample.

73. (Previously presented) The method of claim 72, wherein the calibration comprises generating a serial dilution of the protein sample.

74. (Previously presented) The method of claim 44, wherein analyzing the isolated protein sample further comprises generating on the substrate of the array protein standard comprising a series of at least two dilutions of at least one purified protein.

75. (Previously presented) The method of claim 74, further comprising quantifying at least one protein in the protein sample, where the amount of protein is quantified in units relative to the amount of purified protein in the protein standard on the array.

76. (Currently amended) The method of claim 74, where the protein standard comprises a mixture of two or more purified proteins, ~~each of which~~and wherein each of the two or more purified proteins is used ~~as~~ to calibrate quantification of at least one cellular component in at least one protein sample on the array.

77. (Previously presented) The method of claim 44, wherein each dilution is immobilized within a confined zone that can receive an individual reagent treatment.

78. (Previously presented) The method of claim 59, wherein analyzing the isolated protein sample comprises generating on a substrate an array comprising a series of at least two dilutions of the protein sample.

79. (Previously presented) The method of claim 78, wherein analyzing the isolated protein sample further comprises generating on the substrate of the array protein standard comprising a series of at least two dilutions of at least one purified protein.

80. (Previously presented) The method of claim 79, further comprising quantifying at least one protein in the protein sample, where the amount of protein is quantified in units relative to the amount of purified protein in the protein standard on the array.

81. (Currently amended) The method of claim 79, where the protein standard comprises a mixture of two or more purified proteins, wherein each of the two or more purified proteins ~~which~~ is used ~~as~~ to calibrate quantification of at least one cellular component in at least one protein sample on the array.

82. (Previously presented) The method of claim 78, wherein each dilution is immobilized within a confined zone that can receive an individual reagent treatment.

83. (Previously presented) A method of analyzing the protein content of a population of cells from a tissue sample by screening for the presence of a cellular component in a population of cells from a tissue sample, comprising:

extracting the population of cells from the tissue sample;

isolating a protein sample from the extracted cell population, wherein isolating the protein sample from the extracted cell population comprises lysing the extracted cell population to produce cellular components; and

analyzing the isolated protein sample, wherein analyzing the isolated protein sample comprises:

generating on a substrate an array comprising a series of at least two dilutions of the protein sample;

contacting the array with a binding agent; and

detecting the interaction between the cellular components in the protein sample and the binding agent.

84. (New) The method of claim 1, wherein extracting the population of cells using microdissection under microscopic visualization comprises:

contacting the tissue sample with a transfer film;

focally activating the transfer film with a laser beam, thereby bonding the cells to the transfer film; and

lifting the bonded cells from the tissue sample, thereby extracting the population of cells and leaving unwanted cells behind.

85. (New) A method of analyzing the protein content of a population of cells from a tissue sample, comprising:

contacting the tissue sample with a transfer film;

microscopically visualizing the population of cells in the tissue sample;

focally activating the transfer film with a laser beam, thereby bonding the population of cells to the transfer film;

extracting the population of cells from the tissue sample, thereby leaving unwanted cells behind;

isolating a protein sample from the extracted cell population; and

analyzing the isolated protein sample.

86. (New) The method of claim 85, wherein isolating the protein sample comprises solubilizing the extracted cell contents in less than about 20 µl of a buffer comprising at least one detergent to solubilize the cellular lipids, at least one proteinase inhibitor to preserve protein content and function, and at least one salt to lyse the nuclear contents.

87. (New) The method of claim 86 wherein the cell contents are solubilized in about 1µl to about 15 µl of buffer.